AFRL-AFOSR-UK-TR-2014-0021

Charting the visual space of insect eyes - Delineating the guidance, navigation and control of insect flight by their optical sensor

Doekele G. Stavenga

RIJKSUNIVERSITEIT GRONINGEN
BROERSTRAAT 5
GRONINGEN 9712CP NETHERLANDS

EOARD Grant 12-2053

Report Date: June 2014

Final Report from 1 April 2012 to 31 May 2014

Air Force Research Laboratory
Air Force Office of Scientific Research
European Office of Aerospace Research and Development
Unit 4515, APO AE 09421-4515

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
11 June 2014	Final Report	1 April 2012 – 31 May 2014

4. TITLE AND SUBTITLE

Charting the visual space of insect eyes - Delineating the guidance, navigation and control of insect flight by their optical sensor

5a. CONTRACT NUMBER

FA8655-12-1-2053

5b. GRANT NUMBER

Grant 12-2053

5c. PROGRAM ELEMENT NUMBER

61102F

6. AUTHOR(S)

Doekele G. Stavenga

5d. PROJECT NUMBER

5d. TASK NUMBER

5e. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
RIJKSUNIVERSITEIT GRONINGEN
BROERSTRAAT 5
GRONINGEN 9712 CP NETHERLANDS

8. PERFORMING ORGANIZATION REPORT NUMBER

N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

EOARD
Unit 4515
APO AE 09421-4515

10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/AFOSR/IOE (EOARD)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFRL-AFOSR-UK-TR-2014-0021

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Insect visual systems are extremely compact and presumably optimized for optimal processing of optical information. Unravelling the pathways of spatial, spectral and polarization vision combined in insect eyes and brains will provide essential insight into optimization principles of how animals derive crucial information for survival. In insect compound eyes, the distribution of optical axes determines the spatial sampling of the optical information in the surrounding environment. To map the visual fields of the compound eyes, a motorized goniometric apparatus has been developed allowing the measurement of the visual axes of the sampling units, the ommatidia. With a semiautomated measurement procedure, which uses the prominent pseudopupil phenomenon, spatial maps can be determined. The acquired data forms the basis for analyses of optic flow and optimization of spatial vision depending on speed of flight. The simultaneously acquired data, allowing assessment of ocular heterogeneity, i.e. the dependence of spectral properties on spatial directions, reveals how spatial vision may compete with spectral and polarization vision.

15. SUBJECT TERMS

EOARD, bio-mimicry, insect eye mapping, micro air vehicle control

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18, NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			John Gonglewski
UNCLAS	UNCLAS	UNCLAS	SAR	16	19b. TELEPHONE NUMBER *(Include area code)* +44 (0)1895 616007

[FA8655-12-1-2053] – Charting the visual space of insect eyes

Delineating the guidance, navigation and control of insect flight by their optical sensors

Final report (1 April 2012 – 31 May 2014)

Doekele G. Stavenga
Computational Physics, Zernike Institute for Advanced Materials,
University of Groningen, Groningen 9747AG, The Netherlands

Summary

Insect visual systems are extremely compact and presumably optimized for optimal processing of optical information. Unravelling the pathways of spatial, spectral and polarization vision combined in insect eyes and brains will provide essential insight into optimization principles of how animals derive crucial information for survival. In insect compound eyes, the distribution of optical axes determines the spatial sampling of the optical information in the surrounding environment. To map the visual fields of the compound eyes, a motorized goniometric apparatus has been developed allowing the measurement of the visual axes of the sampling units, the ommatidia. With a semi-automated measurement procedure, which uses the prominent pseudopupil phenomenon, spatial maps can be determined. The acquired data forms the basis for analyses of optic flow and optimization of spatial vision depending on speed of flight. The simultaneously acquired data, allowing assessment of ocular heterogeneity, i.e. the dependence of spectral properties on spatial directions, reveals how spatial vision may compete with spectral and polarization vision.

Introduction

The visual systems of animals sample the light properties of the surrounding world with photoreceptor cells. The spatial distribution of the sampling points, given by the distribution of the photoreceptors' visual axes, appears to be strongly species-dependent, which appears to be intimately related to the animal's behavior and its habitat. Insects have been recognized to be ideal model systems for investigating the spatial characteristics of the visual system because of the enormous variety of insect life styles, habitat, and body sizes. Predatory insects (dragonflies, robberflies) – like predatory higher animals (hawks, falcons, lions) – have distinct foveas, i.e., areas with a high density of spatial sampling points, which are combined with peripheral areas where spatial resolution is coarse. The spatial acuity of non-predatory animals (butterflies, rabbits) is more evenly distributed, but commonly males have an eye area with higher spatial acuity, devoted to mate finding. Spatial eye maps have been partially charted in a few scattered insect cases, but the crucial factors shaping the eyes and their spatial

sampling are poorly known. The aim of this program was to address fundamental issues regarding multisensory contributions to guidance, navigation and flight control (GNC) in a variety of insect species.

Differences in local spatial acuity of insect eyes reflect a functional regionalization that serves different tasks concerning inner-loop control of gaze and flight (low spatial resolution => optic flow processing) and outer-loop, or goal-directed behavior (high spatial resolution => prey/mate detection). Evidently, the way visual axes are organized/packed in a compound eye reflects the different visual tasks an insect has to accomplish and the relative weight it assigns to them. Different regions of the eye feed visual information into pathways specialized for high acuity image segmentation or motion vision, color vision or polarization detection, which may cooperate with other modalities such as mechanosensory and olfactory systems. Quantitative knowledge of the spatial organization of the eyes of various insect species, specialized in a predatory lifestyle or being peaceful visitors of colored flowers, will create insight into the optimization principles of visual image processing.

The visual system performs multiple tasks. In addition to motion and pattern detection, spectral discrimination and color vision are prominent facilities utilized by insects to detect conspecifics for mating, and therefore body coloration can be used for display. Interspecific recognition is complemented by the detection of species-dependent flight-behavior patterns. Alternatively, body patterning and coloration can be optimized for camouflage. As the eye, consisting of the dioptrical apparatus and the retinal layer with photoreceptors, is the input channel of the visual system, detailed knowledge of the primary processing station and its relation of the insect's optical environment is essential for proper analysis of the working of the neural system and of how it mediates successful survival.

Results

In the previous program supported by the EOARD/AFOSR, 'Photonic crystals on the wing' [FA8655-08-1-3012], the research emphasis has been on the optical coloration mechanisms applied by insects. During this highly successful program such a large volume of research data had been accumulated, that its condensation in scientific

publications has taken a protracted time span, much after the official completion of the research project. The decision that it was important to first publish the new insights has so far prevented a fulltime devotion to the 'eye mapping' project. Yet, this was considered to be unavoidable and necessary, because the research on coloration photonics has close links with the planned vision research studies.

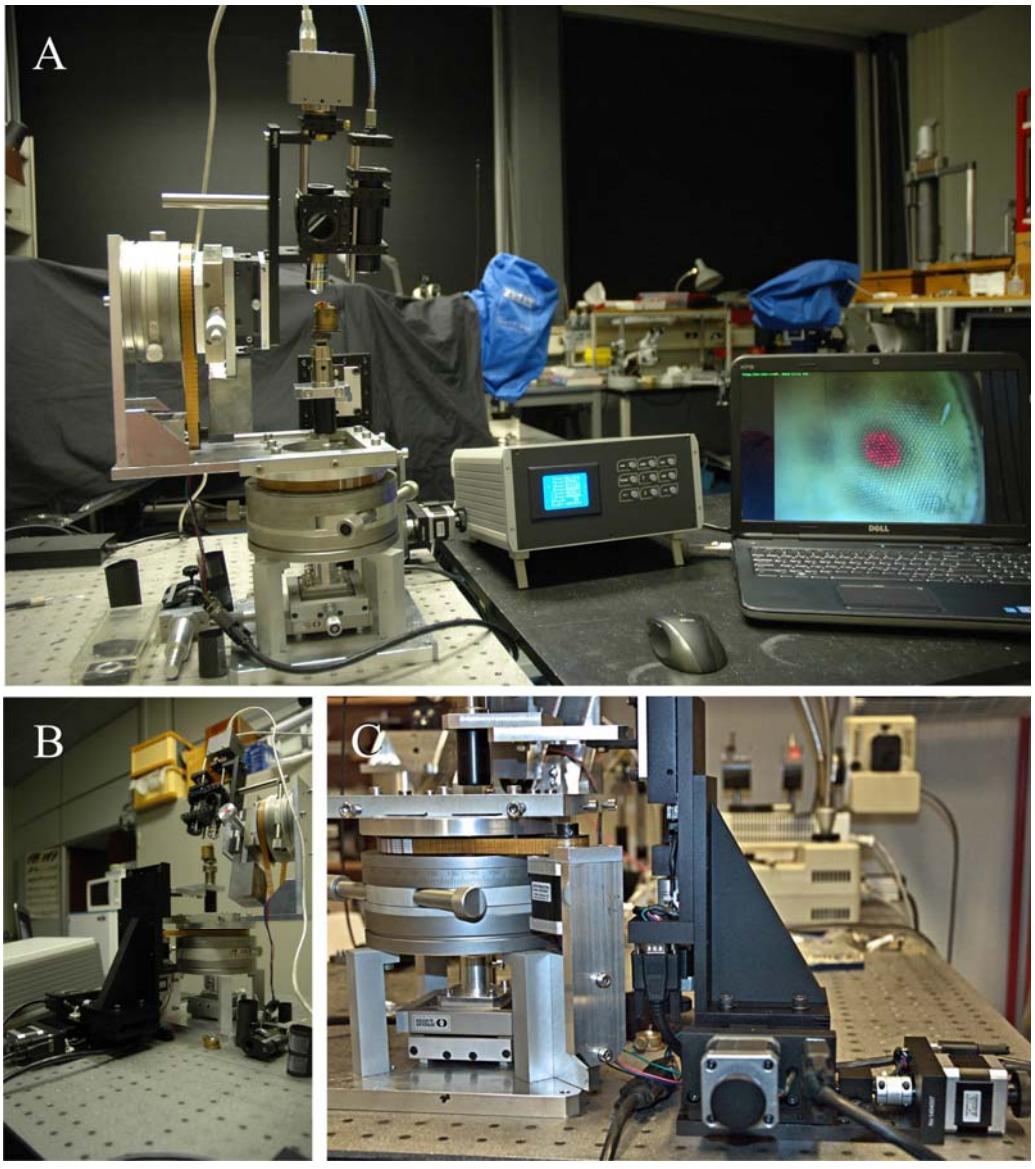

Fig. 1. The eye charting setup. **A** Front view, showing the motorized goniometer with an epi-illumination microscope and camera, the motor driver and PC. **B** Rear view, showing the motorized XYZ-stage with the insect holder. **C** Side view showing the belt-driven horizontal rotator of the goniometer and the the motorized XYZ-stage.

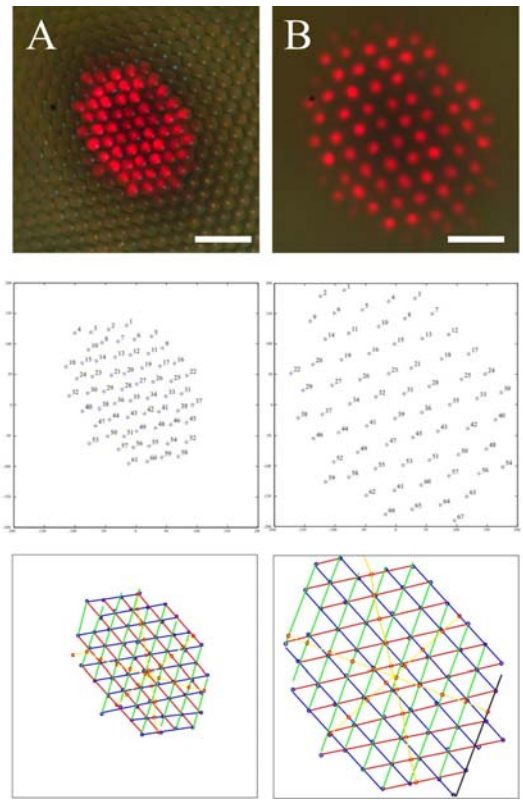

Fig. 2. Images of the eye of a cabbage butterfly and processing of the eyeshine. **A** Image at the corneal level and analysis of ommatidial reflections. **B** Image 1 mm above the cornea and analysis of ommatidial reflections (scale bar: 100 µm).

The present state of the eye charting setup is a motorized goniometer around a holder with the investigated insect on a motorized XYZ-stage (Fig. 1). From pseudopupil images the visual direction of the individual ommatidia are calculated with matlab programs (Fig. 2). Automatization of the analysis procedure has not been realized yet to satisfaction because of limited workforce. We hope to reach that state later this year.

During the program period 22 papers have been produced, which are listed below. The numbered papers can be divided into a few categories:

A. *Butterfly wing coloration*: # 1, 2, 3, 6, 10, 18, 19, 20
B. *Beetle wing colors*: # 4, 5, 8, 13
C. *Damselfly colors*: #7, 12, 20
D. *Insect photoreceptors*: #9, 11
E. *Bird vision and colors*: # 15, 16, 20, 21
F. *Flower colors and insect vision*: # 17, 22

A. Butterfly wing coloration

The wings of butterflies are studded with small scales (size ~150x50x2 μm^3). The scales are generally colored due to pigmentation, but very often they have photonic structures that cause structural coloration. We discovered in the Asian butterfly *Graphium sarpedon* a unique scale type that very approximately acts as an ideal thin film. Oblique illumination results in highly polarized reflections, which presumably mediate interspecific recognition, because butterflies possess polarization vision, in addition to spatial and spectral (color) vision (#1).

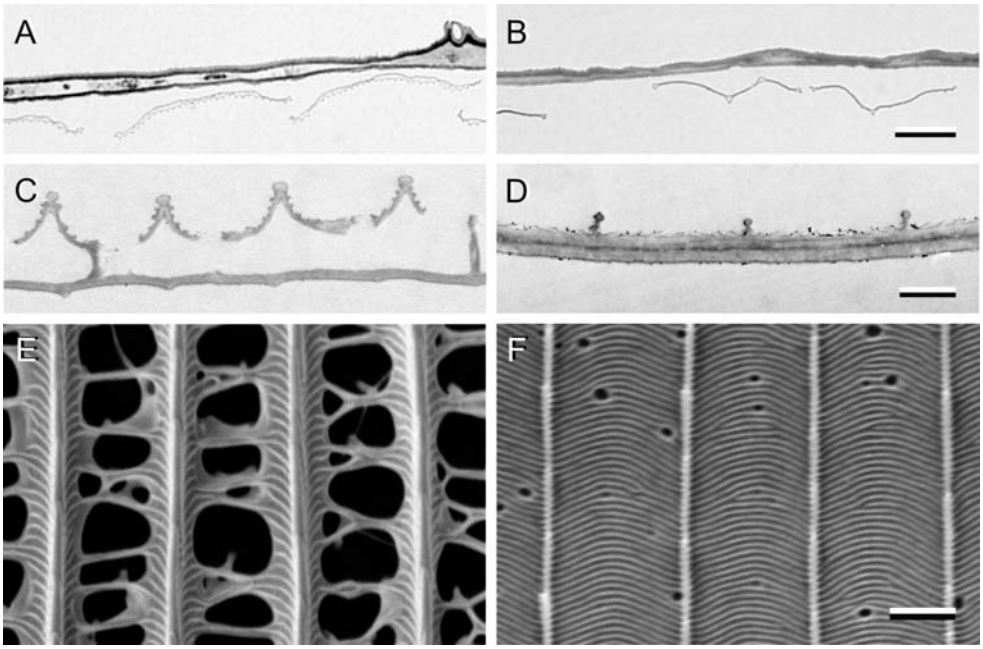

Fig. 3. White and glass scales on the wing under side of *G. sarpedon*. (A) Light microscopical section of a wing patch with a few white scales. (B) A wing patch with a few glass scales. (C) Transmission electron microscopical (TEM) section of a white scale. (D) TEM section of a glass scale (note that the flat, lower lamina faces the wing underside in both C and D). (E) Scanning electron micrograph (SEM) of a white scale. (F) SEM of a glass scale. Bars: 20 μm (A, B), 1 μm (C, D), 1 μm (E, F).

Highly sophisticated structural coloration is realized in certain papilionid butterflies, specifically *Parides sesostris*. The gyroid structures in the wing scales reflect broad-band blue-green light. Interestingly, the structural coloration is tuned to green by blue-absorbing pigment so to enhance camouflage when the butterfly rests on leaves (#3). A similar tuning to a green color occurs in papilionids belonging to the *nireus* group, but

here the structural coloration is caused by highly reflective multilayer structures (#2, 6). A very different method to improve camouflage is practiced by the Japanese angled sunbeam butterfly, *Curetis acuta*. Its wing scales have a bright silvery color, which takes the color of surrounding leaves when the butterfly is at rest (#10).

Recently we discovered that pigmentary tuning of photonic coloration mechanism is almost universal in butterfly wings. Most butterfly wing scales are rather simply structured, with a very thin lower lamina and an upper lamina consisting of ridges connected by crossribs. The color of these scales has so far been generally attributed to the scale's pigmentation. Recently we discovered that the principal coloration tool is the lower lamina, acting as an optical thin film (#18-20). The reflectance band of the thin film is intimately linked to the absorbance spectrum of the pigment (if present). The universal function of pigments in butterfly scales thus is to fine tune the scale's structural reflectance spectrum.

B. Beetle wing colors

Whereas most insect wings are rather thin and flexible chitinous structures, in beetles this holds for only one wing pair; the other two wings are thick and solid curved plates that serve as reliable body covers for sessile or walking beetles. In many beetle species these so-called elytra are extremely reflective, and their optical properties have intrigued numerous scientists, including Newton, Goethe, Raleigh, and Michelson. Our recent studies have clarified various optical mechanisms applied by beetles. The jewel beetles (Buprestidae) have elytra with melanin layers that act as multilayer reflectors, causing brilliant green or red metallic reflections (#13). The Brazilian Diamond Beetle, *Entimus imperialis*, however, has elytra indented by concave pits with structural-colored scales, consisting of domains of three-dimensional photonic crystals that have a diamond-type structure. The domains are very large and thus allowed for the first time to explore the visible-light optical properties of diamond-type photonic crystals (#4) Curiously enough, the sophisticated optics is used to realize camouflaging. Applying additive coloration, the summated reflections of variously colored domains causes an overall green color (#5, 8).

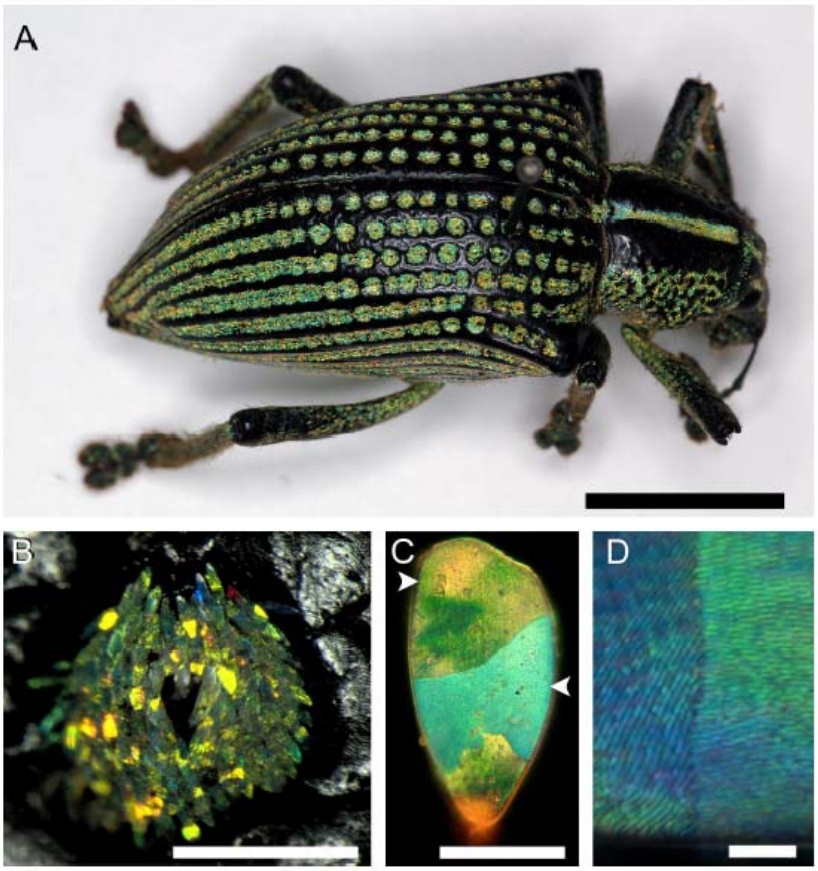

Fig. 4. The Diamond Weevil, *Entimus imperialis*, and its scale organization. **A** The intact animal with the black elytra studded with numerous yellow-green pits (bar: 1 cm). **B** A single pit as seen in an epi-illumination polarization microscope with polarizer and analyzer parallel (bar: 0.5 mm). **C** A single scale with a few differently colored domains. Note the different lamellar arrangements in the yellow and cyan part (white arrowheads, bar: 50 μm). **D** A domain border showing the difference in lamellar arrangements (bar: 5 μm).

C. Damselfly colors

Male broad-winged damselflies (or demoiselles, Calopterygidae) have wing veins with melanin layers that create intense blue wing colors (#7). The coloration could be quantitatively explained using refractive index data obtained with a newly developed polarization-interference method based on Jamin-Lebedeff microscopy (#12). Most dragonflies and damselflies have transparent wings, which are however so thin (< 1 μm) that interference colors arise, which can be understood with thin film optics (#20).

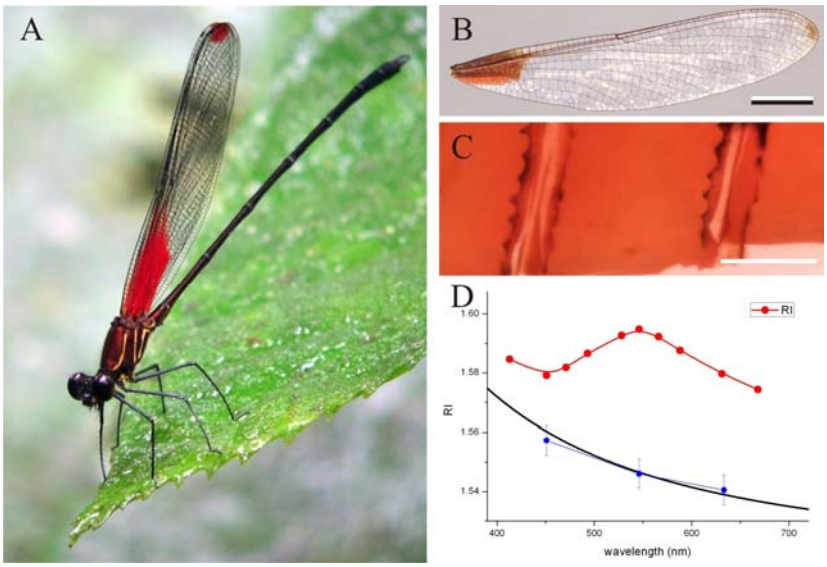

Fig. 5. The American Rubyspot, *Hetaerina americana*. **A** Photograph of the resting damselfly, showing the prominently red pigmented wings. **B** A single wing, with the meshwork of veins (bar: 5 mm). **C** A small section of the red-pigmented wing part, showing that veins as well as the membrane in between are pigmented (bar: 0.1 mm). **D** Real part of the refractive index of the wing membrane as a function of wavelength for the red, pigmented and the clear, unpigmented wing membrane (blue symbols). The black line is the dispersion curve for insect chitin.

D. Insect photoreceptors

Insect vision starts with the absorption of light by the visual pigment (rhodopsin) molecules located in the membrane of the visual photoreceptor cells. The rhodopsin-filled membrane is folded into an optical wave-guiding, cylindrical structure. Previous work has explained in great detail the integrated optical system of facet lenses and optical waveguide. Yet, pigment granules accumulated near the waveguide boundary affects the light flux in the waveguides, thus acting as a colored pupil mechanism, which not only reduces the intensity of the light flux but also its spectral content. The effective spectral sensitivity of the visual photoreceptors hence can be greatly modified. This is especially the case for photoreceptors of pierid butterflies. Together with Japanese colleagues, we have analyzed the spectral sensitivities of the photoreceptors of both male and female Eastern pale clouded yellow butterflies, *Colias erate*. Males and females appear to have different sets of spectral photoreceptors, and therefore their eyes presumably have different color discrimination properties (#9).

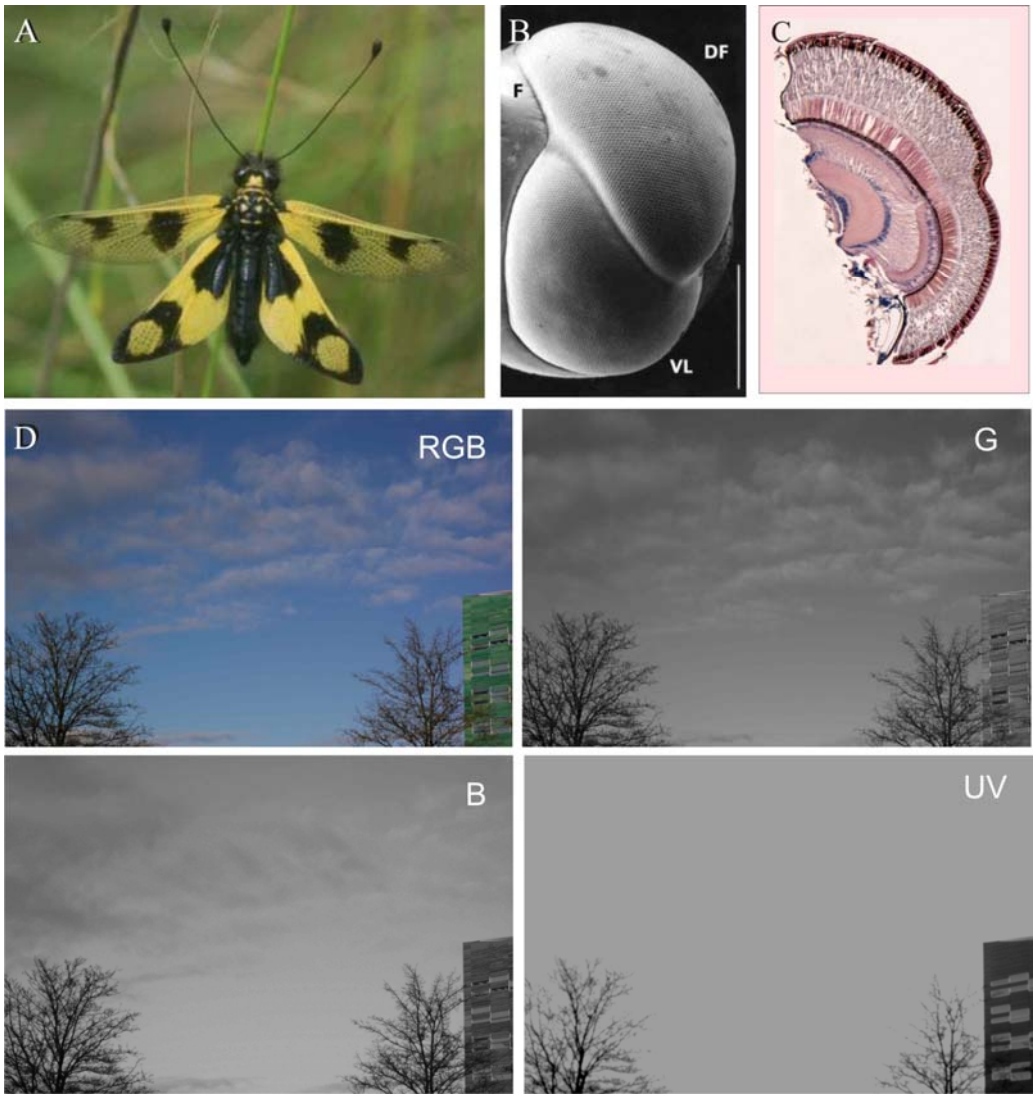

Fig. 6. The owlfly *Libelloides macaronius*. **A** Photograph of the owlfly in a typical resting position, ready to start a flight searching for insect prey. **B** Scanning electron micrograph of the right compound eye, showing the clear sulcus, dividing the dorsofrontal (DF) from the ventrolateral (VL) part. **C** An anatomical section of the eye, showing its superposition structure. **B** The wavelength dependence of the contrast between clouds and sky, indicating the advantage for the owlfly to have virtually exclusively UV sensitive eyes.

In another collaborative study, we have analysed the visual properties of the owlfly *Libelloides macaronius*, which has highly spatially acute vision due to strongly contrast-sensitive photoreceptors (#11).

E. Bird vision and colors

Our studies on spectral filtering of insect photoreceptors initiated a comparative study on bird eyes, where carotenoid-filled oil droplets are positioned in front of the bird cone-type photoreceptors. The oil droplets appear to act as spectral/color filters, but moreover are powerful microlenses. Kramers-Kronig modeling showed that their power depends on the carotenoid concentration, and FDTD (finite-difference time-domain) modeling demonstrated the effective focusing of incident light into the photoreceptor cones (#15).

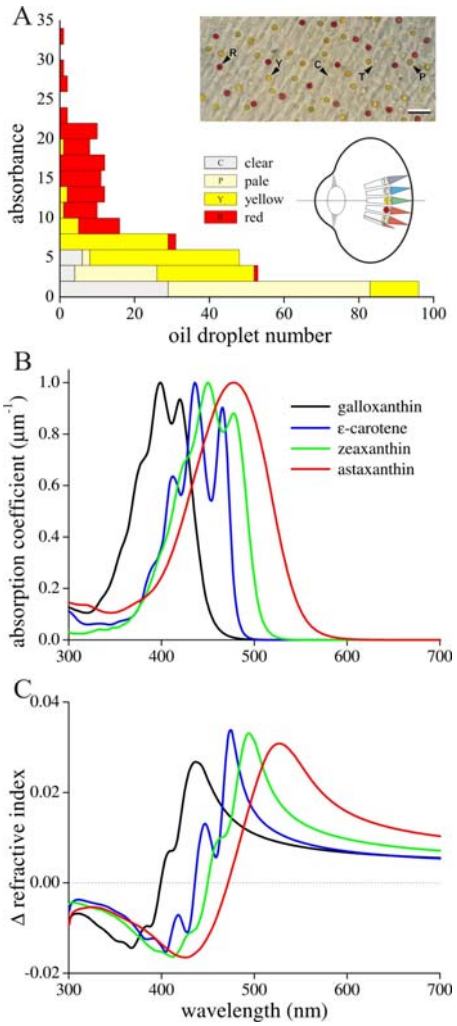

Fig. 7. Oil droplets of bird photoreceptors. **A** The diagram shows a schematic bird eye with the four single cone classes with their oil droplet types (T, transparent; C, clear (or colorless); Y, yellow; R, red), and the double cones with P (pale) droplets. Inset: Section of the retina of an ostrich (*Struthio camelus*); scale bar 10 µm. **B** Absorption coefficients of four carotenoids demonstrated in bird oil droplets, normalized to 1 μm^{-1}. **C** The corresponding contribution to the refractive index of the oil droplets.

FDTD modeling also allowed quantitative explanation of the optics of the spectacularly-colored breast feathers of the bird of paradise Lawes' parotia. The boomerang-shaped barbules of these feathers contain melanin multilayers enveloped by a thin film (#16, 20). The refractive index measurements, essential for the FDTD calculations, were performed on the bird-of-paradise's silver-colored occipital feathers, which act as classical multilayers (#21). A related study has been devoted to the special pigmentation of amazon parrots as well as their structural coloration (14).

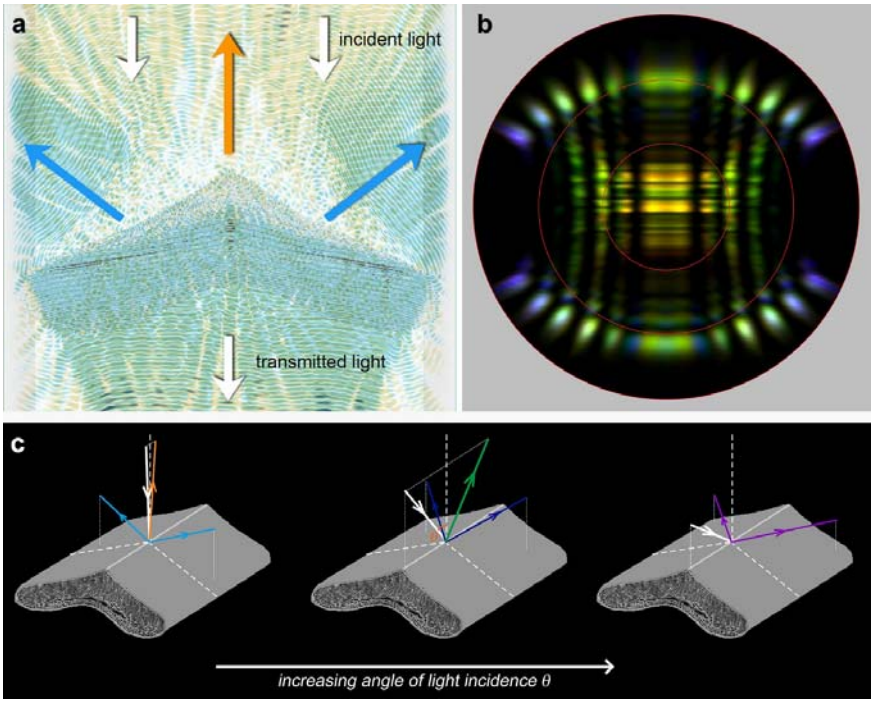

Fig. 8. FDTD modelling of the breast feather barbule of the bird of paradise Lawes' parotia.

F. Flower colors and insect vision

Insects have intimate connections with flowers. We have devoted a detailed study on a recent hypothesis, widely acclaimed, that bees can detect flowers by their iridescence. This should be important for pollination. A crucial tool in our study was our imaging scatterometer, developed 5 years ago. We may emphasize that this unique instrument

could not have been built without the ample support from EOARD/AFOSR. The claimed pollination hypothesis could not be confirmed, and rather it appeared that the theory was unfounded, i.e. built on artificial grounds (#17). The continuation of this work has been a study on the visitation frequency of various insect species on flower communities (#21).

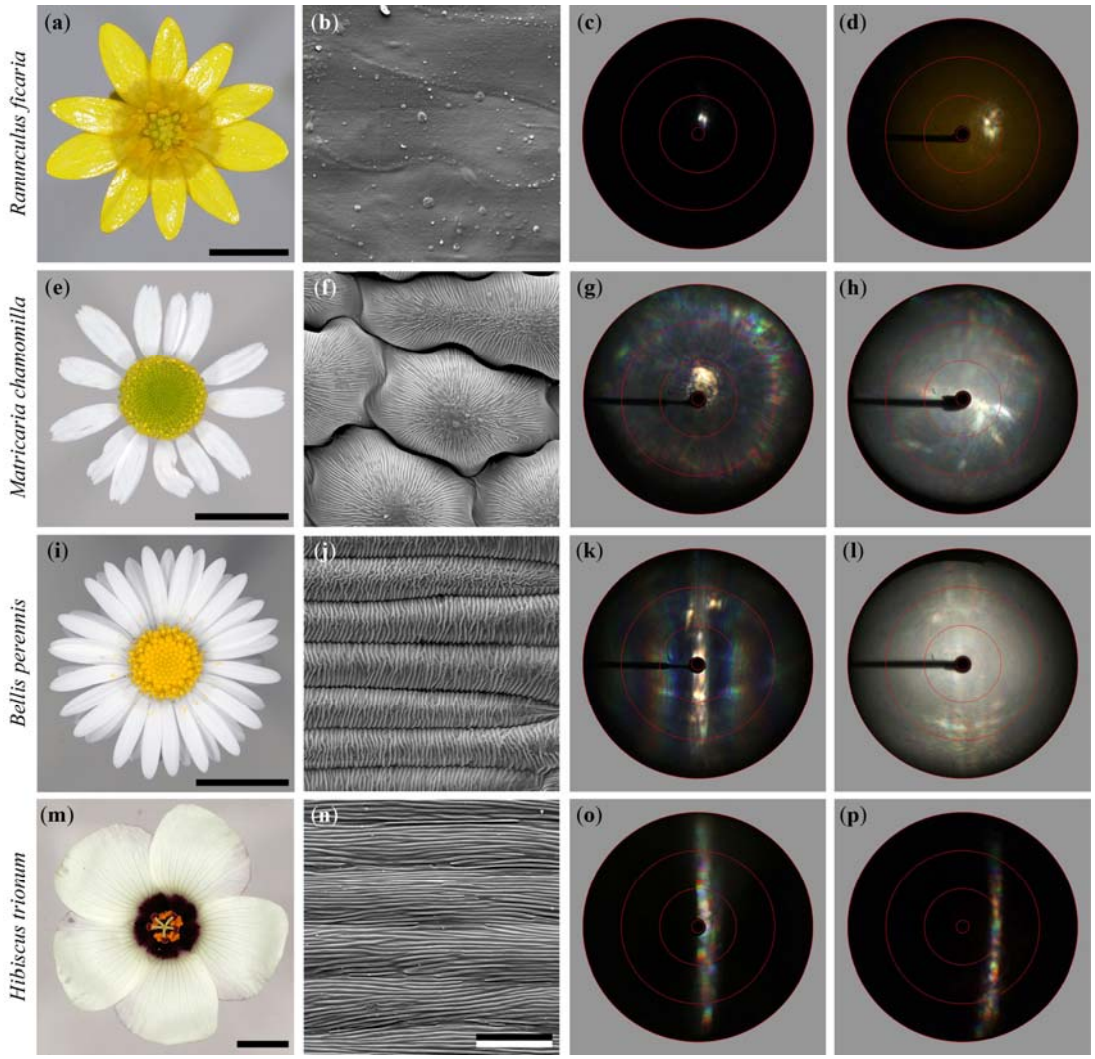

Fig. 9. Flowers with structured petal surfaces and scatterograms. Column 1: habitus pictures of the flowers; column 2: scanning electron micrographs of the flowers' surface structure; column 3: scatterograms of the surface structure of flower casts; column 4: scatterograms of the petals. (a-d) *Ranunculus ficaria*. (e-h) *Matricaria chamomilla*. (i-l) *Bellis perennis*. (m-p) *Hibiscus trionum*. Scale bars: (a,e,i,m) 1 cm, (b,f,j,n) 20 µm. The red circles in the scatterograms indicate angular directions of 5º, 30º, 60º and 90º (see Fig. 2). The black bar at 9 o'clock is due to the sample holder.

Expected developments

The extensive studies on the optics of natural coloration and the interplay of coloration with vision has resulted in a comprehensive description of the main coloration principles underlying animal coloration. We will temporarily continue this research program but the focus is rapidly shifting to the relationship of spectral, spatial and polarization vision. Most importantly, we will finalize the development of the eye charting tool in the near future. We expect that this will become a valuable instrument for the further unravelling of the intricacies of insect vision.

Publications produced during the research period 2012 -2014

2012

1. Stavenga DG, Mashushita A, Arikawa K, Leertouwer HL, Wilts BD (2012) Glass scales on the wing of the swordtail butterfly *Graphium sarpedon* act as thin film polarizing reflectors. J Exp Biol 215:657-662
2. Wilts BD, Trzeciak TM, Vukusic P, Stavenga DG (2012) Papiliochrome II pigment curtails the structural wing colouration of papilionid butterflies of the *nireus* group to the blue-green and thus suppresses iridescence. J Exp Biol 215:796-805
3. Wilts BD, Michielsen K, De Raedt H, Stavenga DG (2012) Iridescence and spectral filtering of the gyroid-type photonic crystals in *Parides sesostris* wing scales. Interface Focus, online, doi:10.1098/rsfs.2011.0082
4. Wilts BD, Michielsen K, De Raedt H, Stavenga DG (2012) Hemispherical Brillouin zone imaging of a diamond-type biological photonic crystal. J R Soc Interface 9:1609-1614
5. Wilts BD, Michielsen K, Kuipers J, De Raedt H, Stavenga DG (2012) Brilliant camouflage – Biophotonic crystals in the scales of the Diamond Weevil, *Entimus imperialis*. Proc R Soc B 279:2524-2530
6. Trzeciak TM, Wilts BD, Stavenga DG, Vukusic P (2012) Variable multilayer reflection together with long-pass filtering pigment determines the wing coloration of papilionid butterflies of the *nireus* group. Opt Express 20, 8877-8890
7. Stavenga DG, Leertouwer HL, Hariyama T, De Raedt H, Wilts BD (2012) Sexual dichromatism of the damselfly *Calopteryx japonica* caused by a melanin-chitin multilayer in the male wing veins. PLoS ONE **7:** e49743.

2013

8. Wilts BD, Ijbema N, K. Michielsen K, H. DeRaedt H, Stavenga DG (2013) Shine and hide: biological photonic crystals on the wings of weevils. MRS Proc. 1504, doi: 10.1557/opl.2013.184
9. Ogawa Y, Kinoshita M, Stavenga DG, and Arikawa K (2013) Sex-specific retinal pigmentation results in sexually dimorphic long-wavelength-sensitive photoreceptors in the eastern pale clouded yellow butterfly, *Colias erate*. J Exp Biol 216:1916-1923

10. Wilts BD, Pirih P, Arikawa K, Stavenga DG (2013) Shiny wing scales cause spec(tac)ular camouflage of the angled sunbeam butterfly, *Curetis acuta*. Biol J Linn Soc 109:279-289
11. Belušič G, Pirih P, Stavenga DG (2013) A cute and highly contrast-sensitive superposition eye – the diurnal owlfly *Libelloides macaronius*. J Exp Biol 216:2081-2088
12. Stavenga DG, Leertouwer HL, Wilts BD (2013) Quantifying the refractive index dispersion of a pigmented biological tissue using Jamin–Lebedeff interference microscopy. Light: Sci Appl 2, e100
13. Schenk F, Wilts BD, Stavenga DG (2013) The Japanese jewel beetle: a painter's challenge. Bioinspir Biomim 8, 045002
14. Tinbergen J, Wilts BD, Stavenga DG (2013) Spectral tuning of Amazon parrot feather coloration by psittacofulvin pigments and spongy structures. J Exp Biol 216:4358-4364

2014

15. Stavenga DG, Wilts BD (2014) Oil droplets of bird eyes: microlenses acting as spectral filters. Phil Trans R Soc B 369, 20130041
16. Wilts BD, Michielsen K, De Raedt H, Stavenga DG (2014) Sparkling feather reflections of a bird-of-paradise explained by finite-difference time-domain modelling. Proc Natl Acad Sci USA, doi/10.1073/pnas.1323611111
17. Kooi CJ van der, Wilts BD, Leertouwer HL, Staal M, Elzenga JTM, Stavenga DG (2014) Iridescent flowers? - contribution of surface structures to optical signalling. New Phytol, doi: 10.1111/nph.12808
18. Stavenga DG, Leertouwer HL, Wilts BD (2014) The colouration toolkit of the Pipevine Swallowtail butterfly, *Battus philenor* – thin films, papiliochromes, and melanin. J Comp Physiol A, doi: 10.1007/s00359-014-0901-7
19. Stavenga DG, Leertouwer HL, Wilts BD (2014) Colouration principles of nymphaline butterflies – thin films, melanin, ommochromes and wing scale stacking. J Exp Biol, doi:10.1242/jeb.098673
20. Stavenga DG (2014) Thin film optics causes structural coloration of many insect wings and bird feathers. Materials Today: Proceedings, submitted May
21. Stavenga DG, Leertouwer HL, Osorio DC, Wilts BD (2014) A dress to impress: ordered melanin layers produce seductively shiny occipital feathers of the bird of paradise Lawes's Parotia. In prep.
22. Kooi CJ van der, Pen I, Stavenga DG, Staal M, Elzenga JTM (2014) Floral colour and the pollination syndrome. In prep.

Groningen, 31 May 2014